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INTERRELATIONSHIPS BETWEEN pH, POPULATIONS OF *PROPIONIBACTERIUM SHERMANII*, LEVELS OF FREE FATTY ACIDS, AND THE FLAVOR RATINGS OF SWISS CHEESES

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SUMMARY

Fifteen experimental cheeses were examined periodically during 1 yr. The following variations in the normal cheeses were found: pH off the press (experimentally induced variation), 4.95–5.37; maximum numbers of *Propionibacterium shermanii*, 145 million/g–2,100 million/g; maximum levels of acids—acetic, 38–59; propionic, 44–104; butyric, 6–40; higher, 22–48 μM/g. With added sodium propionate, pH and numbers of *P. shermanii* increased but production of propionic acid decreased. Five commercial cheeses, examined for flavor and fatty acid levels, typically had higher levels of acetic acid than the experimental cheeses.

Significant relationships were found between pH off the press and growth of *P. shermanii* and also between the maximum levels of propionic acid and both the maximum numbers of *P. shermanii* and the *P. shermanii* numbers-time integrals. All relationships involving the "sweet" flavor component are without significance. With one exception, all relationships involving the "nutty" flavor component are either of doubtful or of no significance. The relationship between nutty flavor and propionic acid levels may be significant.

Propionic acid is a component of the flavor complex, but other compounds appear to be responsible for both the nutty and the sweet characteristics of typical Swiss cheese flavor.

Positive correlations between fatty acid levels and flavor of Swiss cheeses have been reported by several investigators (1, 2, 7, 11, 12). Propionic acid has been considered the most important of the volatile acids in contributing desirable characteristics to Swiss cheese flavor. Babel and Hammer (2) tested 18 strains of propionic acid bacteria and found that, with certain strains, the amount of culture used was correlated positively with the intensity of sweet flavor and the level of volatile acids in the aged cheese. Demeter (3) considered that the propionic acid organisms were very important in the development of flavor in Swiss cheese but that, nevertheless, propionic acid was not the substance responsible for the flavor. Krett *et al.* (10) reported the levels of various fatty acids, but no other values for comparison, during the aging of a typical Swiss cheese.

Frazier *et al.* (5), from a study of over 800 cheeses made under factory conditions, found that the group having a pH of 5.0–5.2 off the press contained the highest percentage of top-quality cheeses. The limit of 5.2 might be exceeded, if the milk were of high quality. Sherman (14) reported that propionibacteria were responsible for the development of characteristic flavor. In a study of cultures, Tittsler (15) found that the optimum pH for growth of *Propionibacterium shermanii* was from 6.0 to 7.0. With increasing distance from the optimum range the initiation of growth was delayed and the rate of growth reduced. If growth occurred, however, the maximum populations reached were the same, regardless of the initial pH.

These various studies have been sufficiently encouraging regarding a correlation between the flavor of Swiss cheese and factors which are susceptible to control in its manufacture, to prompt the present study of the interrelationships between pH, populations of *P. shermanii,* fatty acid levels, and flavor.

EXPERIMENTAL PROCEDURE

Fifteen experimental cheeses, approximately 60 lb. each, were manufactured by a standard procedure (13), except as noted. The starters used were *Streptococcus thermophilus* (S), *Lactobacillus bulgaricus* (Ga), and *P. shermanii* (62). The cheeses were held in the warm room for 4 wk. at a temperature of 72° F. The temperature of the cold room was 50° F. Variations in the pH of the cheeses off the press were experimentally induced by varying the quantity of *S. thermophilus* or *L. bulgaricus,* or by replacing varying amounts of whey with water. A mixture of 1 lb. of propionic acid and 5.9 lb. of sodium propionate was added to the 700 lb. of milk used for making one cheese for which, otherwise, the manufacturing process was normal. Another cheese was made with the addition of the same amounts of propionic acid and sodium propionate as above, but with the omission of the *P. shermanii* starter.

The cheeses were aged for about 1 yr. and examined over this period for flavor, pH, numbers of *P. shermanii,* enterococci, and spore-formers, and for levels of acetic acid, propionic acid, and higher acids as a group. Samples were taken of cheeses off the press and subsequently at intervals varying from 2 wk. for young cheeses to 8 wk. for those 6 mo. or older. Also, pH measurements were made at dipping and at 3 hr. on the press. In addition to the experimental cheeses, four imported cheeses and one domestic commercial cheese, selected for quality, were examined for flavor and fatty acid levels.

Flavor ratings were determined by a panel of experienced judges. Fatty acids were determined by the method of Harper (6), with minor modifications. Ten-milliliter samples of eluate were collected, and meta cresol purple was used as the indicator. In nonaqueous solvents this indicator, in contrast to phenol red, has a pronounced color change near the equivalence point. The titrimetric results are expressed as micromoles per gram of cheese.

The numbers of bacteria were determined in the following manner. They were grown in a medium consisting of 0.5% each of tryptose, tryptone, yeast extract, and sodium lactate and 1.5% agar. Two series of lactate agar plates, with agar seals, were poured for each cheese sample; one was incubated at 45° C. for 3 days and the other at 30° C. for 10 days. This medium, at 30°, supports the growth of propionibacteria but none of the other starter types. During previous research in this laboratory on Swiss cheese it was established that growth on the 45° C. plates is restricted primarily to members of the enterococcus group. Spore-formers, if present, also grow on these plates but are differentiated from the enterococci by colony type. Colonies of propionibacteria begin to appear on the 30° C. plates after 3 days of incubation. They were differentiated from the enterococci by colony type and counted after 10 days of incubation.

RESULTS AND DISCUSSION

Development of P. shermanii. The cheeses were put into the warm room approximately 2 wk. after manufacture. At that time, as expected, none of them contained a large number of *P. shermanii.* The subsequent development varied considerably in the different cheeses. After 2 wk. in the warm room, the populations had increased three to 980 times the corresponding numbers off the press. In 40% of the normal cheeses the maximum populations were reached 6 wk. after manufacture, which coincided with the time of their removal from the warm room. The time at which the maximum populations were reached in the other cheeses varied from 2 to 7 wk. after removal from the warm room. Details of these results are given in Table 1.

TABLE 1

Relation between growth of *Propionibacterium shermanii* and pH off the press

Cheese No.	pH	Population ratio of *P. shermanii*:[b] (No. at 4 wk.)/(No. off the press)	Age of cheese at time of maximum numbers
			(wk.)
1	4.95	3	13
4	4.97	12	10
2	5.00	14	10
5	5.10	10	13
12	5.15	24	6
3	5.20	65	8
14	5.22	60	6
11	5.25	267	6
10	5.35	425	6
6	5.37	980	8
15[a]	5.82	120	8

[a] Sodium propionate was added to the cheese milk.
[b] Represents the number of times the population of *P. shermanii* increased in 4 wk. from the time the cheese was removed from the hoop.

It was found that not only the maximum population of *P. shermanii* but also the period of time during which the population was maintained near the maximum varied considerably from cheese to cheese. In Figure 1, Cheeses No. 6 and No. 11 represent the extremes of the normal cheeses in this respect.

The effect of pH on the development of *P. shermanii* can be considered with respect both to its effect on the maximum populations reached and on the rate of growth when the numbers are appreciably below the maximum. With respect to the latter, it was found, Table 1, that the growth of *P. shermanii* increased directly with an increase in pH of the cheese off the press. The relationship between pH and the growth of *P. shermanii* as represented by the ratio, (numbers of *P. shermanii* at 4 wk.)/(numbers of *P. shermanii* off the press), was closer than any other relationship studied in this investigation. As the initial pH's are all below 6, an increase in pH brings this value closer to the optimum range reported by Tittsler (15). The effect of pH on the early growth of *P. shermanii* in cheese is, therefore, similar to that found in cultures (4, 15).

The maximum population of *P. shermanii* varied considerably from cheese to cheese and, as seen in Figure 4, showed a general positive correlation with pH

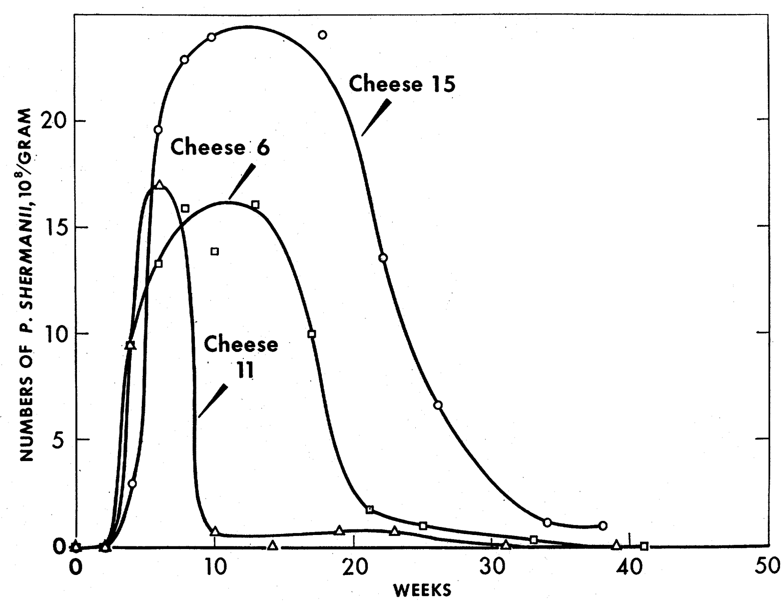

FIG. 1. Development of *P. shermanii* in Swiss cheeses.

off the press. This is in contrast to the behavior of this organism in cultures, as Tittsler found that if *P. shermanii* grew at all the ultimate maximum numbers were the same regardless of initial pH (15).

The correlation between pH and the time needed to reach the maximum population of *P. shermanii* is not as close as that with early growth. This is evident from the data in Column 4 of Table 1. This can be considered to be due to a

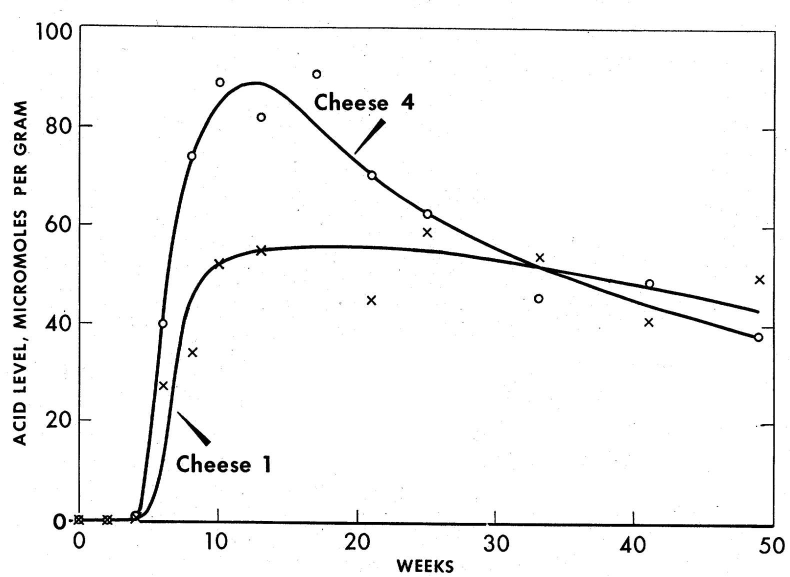

FIG. 2. Development of propionic acid in Swiss cheeses.

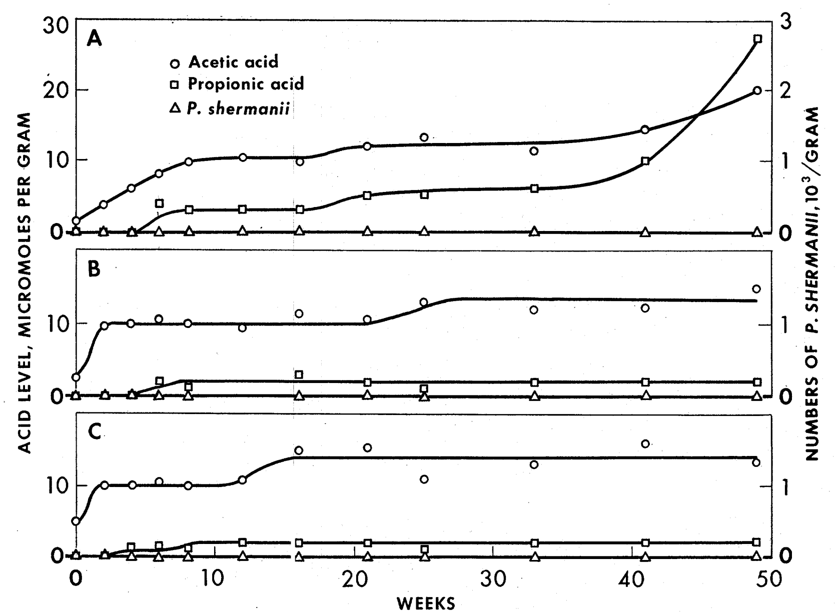

FIG. 3. Development of acids in absence of *P. shermanii*: A, Cheese 7; B, Cheese 9; C, Cheese 8.

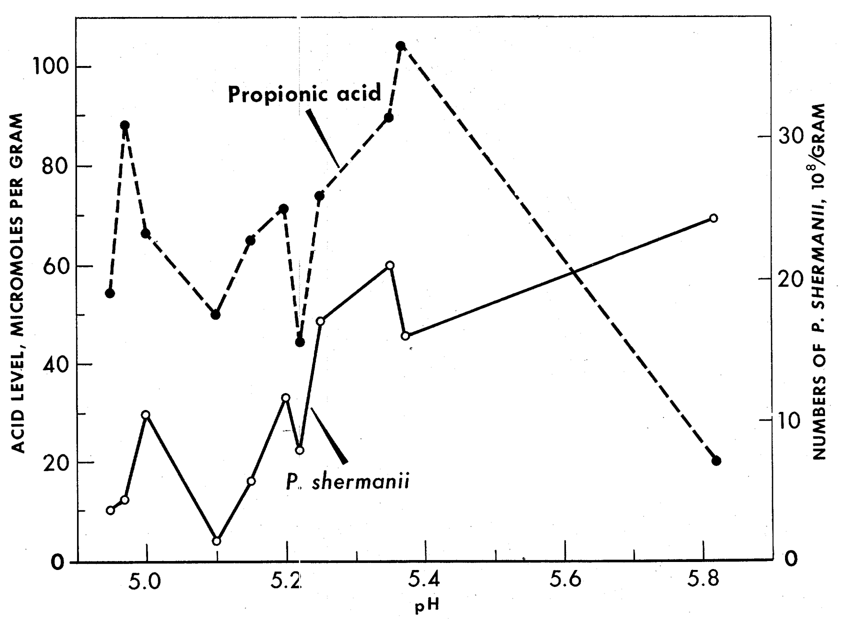

FIG. 4. Interrelationships between pH off the press, maximum numbers of *P. shermanii*, and maximum levels of propionic acid in Swiss cheeses.

modification of the relationship between pH and early growth by the inevitable narrowing of the differences between the population increases as they approach 0 at the maxima and also to the differences in population levels in the various cheeses both at off the press and at their maxima. Cheese No. 6, for instance, with an initial pH of 5.37, reached its maximum population at 8 wk., and Cheese No. 14, initial pH of 5.22, at 6 wk. During these periods, however, the numbers of *P. shermanii* in the former cheese increased more than 16 times as much as in the latter.

Cheese 5, which shows a development of *P. shermanii* somewhat out of sequence with the other cheeses, was also atypical, in that the ratio of acetic acid to propionic acid was exceptionally high. Three of the cheeses, No. 7, 8, and 9, are not included in Table 1. *P. shermanii* could not be detected in these cheeses. However, one of these cheeses, Figure 3, showed the production of considerable propionic acid.

Cheese No. 15, shown in Figure 1, was abnormal in that it was made from milk to which had been added approximately 1% of a mixture of sodium propionate and propionic acid. This mixture reduced the pH of the milk to 5.90. The pH measurements on this cheese at dipping, at 3 hr. on the press and off the press were, respectively, 5.88, 5.88, and 5.82. This seems to indicate that the *S. thermophilus* and *L. bulgaricus* starters were inactivated to such an extent that they did not convert any appreciable amount of lactose to lactic acid. *P. shermanii,* which survived in numbers comparable to those in the normal cheeses, then developed in a lactose rather than in a lactate medium. It can be seen in Table 1 that the growth of *P. shermanii* in this cheese was slower than would be expected from the pH of the cheese. This parallels the finding of El-Hagarawy *et al.* (4), who reported that in cultures this organism grows more slowly in a lactose than in a lactate medium. The maximum number of *P. shermanii* reached in this cheese, 2.4×10^9 per gram, was, however, greater than that of any other cheese. Similarly, El-Hagarawy *et al.* (4) had found that with cultures the slower growth in a lactose medium did not prevent an ultimate population as large as in a lactate medium. The *P. shermanii* in this cheese also had by far the largest numbers-time integral, i.e., the area under the numbers-time curve. However, the production of propionic acid, discussed later, was abnormally low.

Development of other organisms. Spore-formers were generally absent or, if present, rapidly declined in numbers. The data in Table 2 indicate that the non-starter bacteria capable of growth under the plating conditions employed were chiefly enterococci. The likelihood of these organisms being present in large numbers was found to increase both with increasing pH off the press and in the absence of propionibacteria.

Development of fatty acids. The development of propionic acid is illustrated in Figure 2. Generally, the maximum level of propionic acid was found at the same time, or the time of the subsequent analysis, as was the maximum population of *P. shermanii.* In most of the cheeses there appeared to be some loss of propionic acid after the maximum level had been reached. This varied from the extreme

TABLE 2
Mean populations of nonstarter bacteria

Cheese No.	pH off the press	Enterococci[a]	Total bacteria[a, b]
		(thousands per gram of cheese)	
1	4.95	0.4	0.5
4	4.97	0.4	1.1
2	5.00	0.3	0.7
5	5.10	1.1	1.6
8[c]	5.13	1,280	1,620
9[c]	5.13	5,600	7,000
12	5.15	0.1	1.7
3	5.20	0.6	1.8
14	5.22	990	1,150
11	5.25	100	128
7[c]	5.33	202,000	230,000
10	5.35	3.2	5.0
6	5.37	112,000	130,000
13[d]	5.80	730	990
15[e]	5.82	850	940

[a] Mean values of 11 to 13 determinations made over entire ripening period.
[b] As found on lactate agar plate incubated at 30° C. for three days.
[c] No propionibacteria present.
[d] *P. shermanii* omitted; propionate added.
[e] Propionate added.

in Cheese No. 4 to the more typically moderate loss in Cheese No. 1 (both shown in Figure 2) to almost nothing. The loss for each cheese can be determined from the data in Table 4. The significance of curves such as those in Figure 2, in attempting to correlate flavor with propionic acid levels, is discussed later. The cause of such a high loss of propionic acid as that found in Cheese No. 4 is not known, but in this particular cheese it was associated with an extremely poor texture which was cabbagy, with dry, slit-like eyes.

The three parts of Figure 3 pertain to the three cheeses of one day's manufacture. The complete absence of detectable organisms of the genus *Propionibacterium* in each of the three cheeses would appear to be explained most readily by assuming an inadvertent omission of the *P. shermanii* starter. The small level of propionic acid in Figures 3B and 3C is within the experimental error of its determination. The significant level of this acid, shown in Figure 3A, would appear to be an unusual instance of the formation of propionic acid in Swiss cheese by organisms other than those of the genus *Propionibacterium.* So far as the authors are aware, this has not been previously reported with substantiation.

The ratios of propionic to acetic acid found in the various cheeses are given in Table 3. Johns (8), working with cultures of *P. shermanii,* found that with increased carbon dioxide tension in the medium the ratio varied from 1.76 to 3.56 in glucose media but from only 1.90 to 2.14 in lactate media.

At 2 wk. after manufacture, before the development of appreciable propionic acid, acetic acid was invariably found to be present in the cheeses. Figure 3 shows that acetic acid, in comparable concentrations, was produced also in those cheeses in which *P. shermanii* could not be detected. It would thus appear that the acetic acid levels found in Swiss cheese result not only from the activity of *P. shermanii* but from that of other organisms as well.

TABLE 3

Ratios of propionic acid to acetic acid in Swiss cheese

Cheese No.	Ratio as found in cheese	Ratio after correction for initial production of acetic acid
1	1.5	2.4
2	1.4	1.8
3	1.3	1.6
4	1.6	2.2
5	1.1	1.7
6	2.0	2.1
7[a]	1.5	2.0
8[a]	0.1	....
9[a]	0.2	....
10	2.1	2.2
11	1.6	2.0
12	1.5	2.0
13[b]	0.0	....
14	1.0	1.6
15[c]	1.3	1.7
16[d]	1.0	....
17[d]	0.9	....
18[d]	1.0	....
19[d]	0.8	....
20[e]	0.8	....

[a] *P. shermanii* could not be found in this cheese.
[b] Sodium propionate was added to the cheese milk—*P. shermanii* was omitted.
[c] Sodium propionate was added to the cheese milk.
[d] Imported commercial cheese.
[e] Domestic commercial cheese.

TABLE 4

Free fatty acids and flavor of Swiss cheeses

Cheese No.	Age	Propionic	Acetic	Butyric	Higher	Sweet	Nutty	Off-flavor
	(wk.)	*(μM per gram)*						
5	49	29 (50)[a]	34	6	18	Medium	Absent	Absent
4	49	38 (89)	35	4	16	Slight	Absent	Absent
1	49	43 (55)	35	7	24	Medium	Absent	Absent
14	24	44 (44)	40	34	24	Slight	Absent	Malty and dirty
3	49	45 (72)	33	6	31	Slight	Absent	Absent
20	....	48	58	6	35	Slight	Slight	Acid
17[b]	....	51	59	3	17	Medium	Medium	Absent
2	49	53 (67)	37	4	24	Intense	Absent	Absent
19	....	57	72	3	17	Slight	Absent	Absent
12[b]	39	64 (66)	42	5	19	Medium	Medium	Absent
16	....	66	68	2	16	Absent	Absent	Absent
11[b]	39	72 (75)	45	5	33	Medium	Medium	Absent
18	....	77	75	3	13	Medium	Medium	Dirty
6	39	89 (104)	44	11	32	....	....	Rotten
10	39	90 (90)	43	6	44	Medium	Slight	Absent

[a] Parenthetical values are maxima. All other data pertain to samples taken at time shown in Column 2.
[b] Grade A cheese.

Column 2 of Table 3 lists the (propionic acid)/(acetic acid) ratios as actually found in the cheeses. Column 3 of this table lists these ratios after they have been corrected by subtracting the acetic acid level at 2 wk. from the maximum level of acetic acid reached at a later date. This is a reasonable way to account for the acetic acid produced by organisms other than *P. shermanii*. Even though such a correction must be rather inaccurate, it is clear that the corrected values are much closer than the uncorrected to the theoretical value of 2.0.

The commercial cheeses, both domestic and imported, were typified by high levels of acetic acid with the (propionic acid)/(acetic acid) ratios varying from 1.0 to 0.8. In addition to the carbon dioxide tension, Johns (8) has listed the initial and final pH of the medium, the strain of *Propionibacterium,* and the nature of the substrate as factors which would influence the (propionic acid)/(acetic acid) ratio. Information is not available as to whether the comparatively high levels of acetic acid in the commercial cheeses arose from the influence of any of these factors or were due entirely to the activity of organisms other than those of the genus *Propionibacterium.*

The levels of butyric acid and of the higher acids, at the time of the final examination of the cheeses, are given in Table 4. In general, the final levels of these acids are nearly the same as their maximum levels. The two cheeses containing added sodium propionate, not listed in this table, had the abnormally high butyric acid levels of 29 and 51 $\mu M/g$.

Flavor. It can be seen from the comments in Table 4 that the most frequent flavor defect of the cheeses was a lack of the quality described as nutty. Of ten normal experimental cheeses, this quality was absent in six, present in three, and unjudgable in one because of a pronounced off-flavor. In two of the five samples of commercial cheeses, the nutty quality was completely lacking. The sweet quality of Swiss cheeses flavor, in contrast, was completely lacking in only one of 14 cheeses. Off-flavors were found in four of the 15 cheeses.

Interrelationships. The relationship between the development of *P. shermanii* and pH has already been discussed. The interrelationships between these values and maximum levels of propionic acid can be seen in Figure 4. The production of propionic acid was just about as closely related to the *P. shermanii* numbers-time integrals as to the population maxima (Table 5).

Babel and Hammer (1, 2) thought that propionates in Swiss cheese were sweet and contributed directly to the intensity of the sweet flavor. Pette (12) also thought that propionates were directly responsible for sweetness in Swiss cheese. Krett and Stine (11) and Hintz *et al.* (7) found a positive correlation between the levels of propionates in Swiss cheeses and the intensity of characteristic flavor. These investigators, however, were noncommittal as to whether the propionates contributed directly to the characteristic flavor or whether they were correlated with the levels of some other substance or substances which were the flavor contributors.

If the former were true, the flavor ratings of a series of cheeses should show a positive correlation with the levels of propionic acid in the cheeses at the time of grading. In the latter case, since it is unlikely that the levels of the flavor contributor would be affected by aging in exactly the same way as would those of propionic acid, observed correlations between flavor ratings and propionic acid levels would be affected by the age of the cheeses. The correlations would be affected adversely by the extent to which the cheeses under comparison differed in the percentage loss of propionic acid with aging, and might be closest at the time of maximum levels of propionic acid.

Aqueous solutions of propionic acid and propionate ion (as sodium propionate) in proportions and total concentrations found in Grade A cheeses were tested for flavor organoleptically. The solutions were devoid of either a sweet or a nutty characteristic, but were salty and had a quality which could be described as rich and which was reminiscent of Cheddar rather than of Swiss cheese. However, the flavor of propionic acid might be modified in the complex environment of cheese. Therefore, its flavor in water is evidence, but not proof, that it is not the contributor of either the sweet or the nutty characteristic of Swiss cheese. The data in Table 4 also indicate that the sweet characteristic, at least, is unrelated to the propionic acid level. Propionic acid appears to be neither the sweet flavor component, nor an indicator of its presence. The relationship between the nutty characteristic and propionic acid is discussed below.

Hintz *et al.* (7) reported that a minimum propionic acid concentration of 5.0 mg. per gram of cheese was necessary before the cheese possessed a satisfactory Swiss cheese flavor. This is equivalent to 67 μM/g. The levels of propionic acid found in the two experimental Grade A cheeses are very close to this value. However, the concentration of 51 μM of propionic acid per gram of cheese found in the imported Grade A cheese would indicate that the minimum value reported in the literature can not be considered to be a general requirement for Swiss cheese of satisfactory flavor.

Propionic acid is flavorful and, therefore, is a component of the flavor complex. It would appear, however, that other compounds are responsible for both the nutty and the sweet characteristics of typical Swiss cheese flavor. Taken as a whole, the analyses indicate that it is much more difficult to manufacture Swiss cheese with a satisfactory level of characteristic flavor than with a satisfactory level of propionic acid.

Numerical evaluation of the interrelationships has been made by the methods described by Kendall (9). The results are given in Table 5. The correlation coefficient, τ, can vary from $+1$ through 0 to -1. This is true whether the qualities whose relationship is being tested are dependent or independent. If independent, however, the value of τ will most probably be near to 0 and least probably near to ± 1. The probability, P, that a value of τ can have been obtained from a relationship between independent qualities can, therefore, be used as a test of the significance of τ. The lower the value of P, which is variable between 0 and 1, the greater is the significance of τ and the greater the probability that the relationship is between dependent qualities. The choice of an exact value of P for considering τ significant must be arbitrary. A value of 0.05 or less, however, is often considered sufficiently low to justify an assumption that a relationship is significant.

TABLE 5

Various relationships in Swiss cheeses

Relationship	τ	P
$\left[\dfrac{\text{No. of } \textit{Propionibacterium shermanii} \text{ at 4 wk.}}{\text{No. of } \textit{P. shermanii} \text{ off the press}}\right]$ and pH off the press	.87	$.0^{3}1$
P. shermanii maxima and pH off the press	.64	$.0^{2}9$
P. shermanii maxima and propionic acid maxima	.56	.03
P. shermanii no.–time integral and propionic acid maxima	.51	.05
Propionic acid maxima and pH off the press	.38	.16
Sweet flavor and propionic acid content	.12	.59
Nutty flavor and propionic acid content	.47	.05
Sweet flavor and propionic acid maxima	.04	1.00
Nutty flavor and propionic acid maxima	.30	.38
Sweet flavor and *P. shermanii* maxima	.04	1.00
Nutty flavor and *P. shermanii* maxima	.37	.26
Sweet flavor and pH off the press	−.10	.81
Nutty flavor and pH off the press	.45	.17
Sweet flavor and *P. shermanii* no.–time integral	.10	.81
Nutty flavor and *P. shermanii* no.–time integral	.22	.53

Using this test of significance, the data in Table 5 indicate the following: The relationships between pH off the press and both the growth and maximum numbers of *P. shermanii* are highly significant. The relationships between the maximum levels of propionic acid and both the maximum numbers of *P. shermanii* and the *P. shermanii* numbers-time integrals are significant. The correlation between maximum levels of propionic acid and pH off the press is not high enough to exclude the possibility that the relationship could have arisen by chance.

There is no significance to any of the relationships involving the sweet component of Swiss cheese flavor. With one exception, all the relationships involving the nutty flavor component have correlation coefficients which are too low to justify the assumption of significance. The exception is the relationship between the nutty flavor component and the propionic acid level at the time of judging. The probability of only 0.05 that the observed relationship could have occurred by chance is sufficiently low to warrant the assumption of significance. However, organoleptic tests indicated that it is improbable that propionic acid, itself, is the nutty flavor component. Also, if the acid acted as an indicator of nutty flavor, one would expect a closer correlation than was found between the maximum level of the acid and nutty flavor. Thus, it is felt that this relationship is best described by saying that it may be significant.

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